Sensitivity of Larvae, Pupae, and Adults of the Driedfruit Beetle (Coleoptera: Nitidulidae) to Gamma Radiation[1]

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ABSTRACT Raisins infested with three different ages of larvae of driedfruit beetle, *Carpophilus hemipterus* (L.), were treated with gamma radiation at doses of 130–798 Gy. Pupae and unmated adults were also treated at 338 and 486 Gy. All doses to larvae prevented adult development. Only the oldest treated larvae were able to develop to the wandering stage (late third instar) before dying. Complete larval mortality occurred sooner at higher doses. Most younger larvae died within the raisins. Mortality of irradiated pupae at both doses was 90%, and adults emerging from treated pupae died within 48 h. Irradiated adults produced no progeny and died within 1 wk after treatment.

KEY WORDS Insecta, *Carpophilus hemipterus*, raisins, disinfestation, irradiation

THE DRIEDFRUIT BEETLE (DFB), *Carpophilus hemipterus* (L.), is a frequent pest of ripening and drying fruit and is a problem in California's raisin industry. Adults are attracted to damaged and rotting grapes that have been set out to dry. Damage to the drying grapes is increased by feeding of the beetles and their larvae; raisin quality is reduced by contamination with feces and exuvia (Soderstrom et al. 1982). High fruit moisture levels are normally needed for infestation, but larvae can continue development as the fruit dries (Simmons & Nelson 1975). Late third instars, known as wandering stage larvae (Hall et al. 1978), enter the soil where they pupate in earthen cells.

Raisins brought from the field are normally fumigated with methyl bromide or hydrogen phosphide to control DFB and other insect infestations, and additional treatments may be required while the raisins are in storage. Concern over the possible loss of the use of chemical fumigants has prompted research into alternative methods for dried fruit disinfestation. Recent approval by the U.S. Food and Drug Administration of low-dose (≤1 kGy)[2] ionizing radiation treatments of fruits and vegetables (Young & Bowen 1986) has made available the use of radiation as a substitute for fumigation.

Research on the efficacy of irradiation in controlling postharvest insect pests has included a number of different coleopterous species, but little research has been done with nitidulids (Tilton & Burditt 1983). Papadopoulou (1964) used relatively high doses (1.0–1.5 kGy) to obtain mortality in larvae and adults of DFB. Brower et al. (1973) prevented adult development from eggs and larvae of the corn sap beetle, *Carpophilus dimidiatus* (F.), with doses as low as 50 Gy and significantly reduced longevity of adults treated with 500 Gy.

To determine the efficacy of ionizing radiation for control of DFB infestations in dried fruit, the effect on as many stages as possible should be considered because any stage may be present during treatment. The objective of my study was to examine the effect of gamma radiation on DFB larvae infesting raisins, and on DFB pupae and adults.

Materials and Methods

All test insects were obtained from laboratory cultures reared on figs. Dried figs were soaked in water overnight and added to glass canning jars (0.95 liter) filled halfway with moist, sterile sand. Adult beetles were transferred to the jars and allowed to feed and oviposit for 1 wk. Jars containing infested figs were held for DFB larval development and subsequent adult emergence.

For irradiation of larvae, glass canning jars (0.48 liter) were used as test units. The lid of each jar was replaced with filter paper (9.0 cm diameter) overlying copper screen (40 mesh). A thin layer of plaster of paris (ca. 2 cm thick) was poured into the bottom of each jar and allowed to harden. All jars were sterilized with steam before use. The day before infestation, the plaster of paris was saturated with sterile deionized water. Raisins (125 g) were added to each jar and allowed to rehydrate overnight.

A measured volume of adult DFB known to approximate 600 beetles was used to infest the raisins

[1] This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or a recommendation for its use by USDA.

[2] The Systeme Internationale (SI) unit for expressing absorbed radiation is the Gray (Gy), which replaces the older, more familiar rad. One kilorad (krad) = 10 Gy.

in each of 25 test jars. Adults were allowed to oviposit for 72 h before transfer to new jars. Such transfers were made three times, resulting in three age classes of larvae. The 25 jars containing larvae of each age class were randomly divided into five treatments of five replicates each.

Test insects were irradiated at the Sandia National Laboratories in Albuquerque, N. Mex. The day before irradiation of DFB larvae, paper toweling was loosely packed on top of the raisins in each jar to reduce shifting and prevent injury to the larvae during transit. Jars were then wrapped in plastic foam sleeves and packed in insulated plastic ice chests for overnight shipment by air freight to Albuquerque. Larval age at the time of irradiation was 1–4, 4–7, and 7–10 d for the three age groups. A cesium-137 prototype sludge irradiator (Morris 1980) with a dose rate of ca. 15 Gy/min was used to apply doses of 130, 287, 563, and 798 Gy to the test units. Actual doses were determined by placing dosimeters (TLD-400 [LiF]) within test jars. Jars containing control larvae (0 Gy) were also shipped, but left untreated.

All test insects were returned to Fresno, Calif., immediately after irradiation. Jars containing raisins infested with larvae were held at 27°C until larvae were judged to be near maturity (18 d after infestation). Jar lids were replaced with coarse screen and the jars were inverted over food cartons (0.24 liter) containing a thin layer of moist sand and banana slices. The sand was examined periodically for mature larvae, pupae, and adults. After 3 d, the raisins were examined for any remaining living DFB larvae; these were added to those in the sand. All recovered DFB were kept on the moist sand until development was complete or mortality occurred. Raisins were frozen and stored for later extractions of dead DFB.

Wildman trap flasks were used to extract dead DFB from test raisins with a procedure modified from Horowitz (1980). To make the trap flask, a rubber stopper (no. 8½) attached to a metal rod was forced into an Erlenmeyer flask (1.0 liter). Thawed raisins from one test jar were added to the flask, and the jar was rinsed twice with 25% EtOH. The jar rinsings were added to the flask with enough 25% EtOH to bring the volume to ca. 600 ml. A 30-ml amount of flotation oil (mineral oil and kerosene in a 2:1 mixture) was next added and thoroughly stirred into the raisin and alcohol mixture with the rod and stopper. Enough 25% EtOH was then added to bring the fluid level well into the neck of the flask. The raisin layer was stirred intermittently for 20 min on a magnetic stir plate and then allowed to sit undisturbed for another 10 min. The oil layer was trapped off by bringing the rubber stopper up into the neck of the flask. The trapped liquid was filtered with suction through a Büchner funnel lined with ruled filter paper. Beginning with the addition of 30 ml of flotation oil, the procedure was repeated and the resulting oil layer was trapped off and filtered. The filter paper was examined under a dissecting scope and all DFB were removed and placed in 70% EtOH. Larval head capsules were measured to estimate instar distribution.

Table 1. Mean survival of three different ages of DFB larvae irradiated in raisins

Larval age at irradiation (days)	Dose (Gy)	$\bar{x}$ no. DFB recovered 21 d after infestation		$\bar{x}$ no. DFB surviving to adult stage
		Living	Dead	
1–4	0	44.6	2.0	42.6
	130	0	0	0
	287	0	0	0
	563	0	0	0
	798	0	0	0
4–7	0	29.0	0.2	27.8
	130	0	2.6	0
	287	0	4.0	0
	563	0	0	0
	798	0	0	0
7–10	0	40.8a	0.2a	39.0
	130	27.4b	18.2b	0
	287	21.4b	43.8c	0
	563	1.4c	45.8c	0
	798	0c	34.4c	0

Means within columns followed by the same letter are not significantly different ($P > 0.05$; Duncan's [1955] multiple range test).

For irradiation of pupae and unmated adults, prepupae and pupae were separated from the sand substrate of colony jars by flotation and collected on a 20-mesh sieve. Newly formed pupae were obtained by holding prepupae at 27°C in petri dishes (9.0 cm diameter) lined with moist filter paper. Pupae were removed daily and transferred to similar dishes held at 10°C. Pupae were kept at 10°C for 1–3 d until sufficient numbers were available for testing. The day before irradiation pupae were segregated by sex into polystyrene automatic analyzer beakers (2 ml, dispo, American Scientific Products, Sunnyvale, Calif.) and covered with moist vermiculite. Twenty-five pupae were placed in each beaker and eight beakers for each sex were used at each of three treatments.

Pupae removed from colony jars were segregated by sex and held at 27°C for adult emergence. Ten newly emerged adults of one sex were placed in analyzer beakers (2 ml) containing folded paper strips. The inner walls of each beaker were streaked lightly with honey. Five beakers for each sex were used for each of three treatments.

Beakers containing pupae and adults were pressed into styrofoam blocks and packed in ice chests for overnight shipment to Albuquerque. Treatment doses of 338 and 486 Gy were applied in a cesium-137 chamber irradiator with a dose rate of ca. 12.5 Gy/min. Irradiated and untreated control beakers were returned to Fresno immediately after treatments.

Replicates of male and female adults within each treatment level were paired and placed in jars (0.48 liter) containing moist, sterile sand and banana slices. The jars were checked periodically for adult

Table 2. Instar distribution of DFB larvae extracted from irradiated raisins

Larval age at irradiation (days)	Dose (Gy)	$\bar{x}$ no. larvae per instar			Total
		1st	2nd	3rd	
1–4	0	0	0	0.6	0.6a
	130	15.4	9.4	3.0	27.8b
	287	11.8	5.6	0.2	17.6b
	563	18.0	10.8	0.6	29.4b
	798	16.0	7.4	0.2	23.6b
4–7	0	0	0.4	0.2	0.6a
	130	1.8	3.2	8.6	13.6ab
	287	1.2	8.0	15.2	24.4bc
	563	5.0	22.0	11.2	38.2c
	798	1.8	21.4	16.2	39.6c
7–10	0	0.2	0.6	0.8	1.6a
	130	0.8	0.4	2.6	3.8a
	287	0.6	2.4	0.8	3.8a
	563	0.4	0.6	4.2	5.2a
	798	0.4	4.8	20.2	25.4b

Means within columns followed by the same letter are not significantly different ($P > 0.05$; Duncan's [1955] multiple range test).

mortality and progeny development. Test beakers containing pupae were examined daily for adult emergence.

Data were subjected to analysis of variance; arcsine transformations were used on percent mortality of irradiated pupae. Data from different larval age classes were analyzed separately. Significant ($P \leq 0.05$) treatment means were separated by Duncan's (1955) multiple range test.

Results and Discussion

The presence of living or dead DFB in the sand underneath test jars containing the oldest larvae (7–10 d old when irradiated) indicated that the larvae developed to the wandering stage and exited the raisins in search of pupation sites (Table 1). Most of the irradiated larvae died before pupation. One larva irradiated with 287 Gy successfully pupated, but was not able to complete development. At 21 d after infestation, the percentage of dead larvae recovered from the sand increased with increasing doses, suggesting that higher doses caused earlier mortality.

None of the middle-aged larvae (4–7 d old when irradiated) were recovered alive from irradiated raisins 21 d after infestation, although a few survived to the wandering stage at the lowest doses (Table 1). No larvae, alive or dead, were recovered from the sand underneath irradiated raisins containing the youngest larvae (1–4 d old when irradiated).

These results are corroborated by those from the raisin extractions (Table 2). Few DFB larvae were extracted from any of the untreated raisins, but large numbers were found in irradiated raisins containing young and middle-aged larvae. From raisins infested with older larvae, large numbers of larvae were extracted only from those irradiated with 798 Gy. Instar distribution of extracted larvae, determined by head-capsule measurements, showed that most DFB irradiated as young larvae died in the first or second instar, whereas those treated as middle-aged larvae died as second or third instars (Table 2). Thus, only larvae irradiated when 7–10 d old reached the wandering stage and exited the raisins. Younger larvae died at an earlier developmental stage and remained within the raisins.

DFB pupae and adults also were relatively radiosensitive. Pupal mortality was 91.5 and 98% for 338 and 486 Gy, respectively, with no differences due to sex of the pupae (Table 3). Adults emerging from irradiated pupae died within 48 h after emergence.

Table 3. Mortality of DFB irradiated as pupae

Dose (Gy)	% mortality	
	♀♀	♂♂
0	19.5a	20.1a
338	91.5b	91.5b
486	98.0b	98.0b

Analysis of variance ($n = 200$) with arcsine transformation. Values given are untransformed means. Means in a column followed by the same letter are not significantly different ($P > 0.05$; Duncan's [1955] multiple range test).

Table 4. Mortality of DFB irradiated as adults

Dose (Gy)	% mortality			
	1 d posttreatment		8 d posttreatment	
	♀♀	♂♂	♀♀	♂♂
0	16.0a	18.9a	42.0a	26.0a
338	30.0a	32.0a	100b	100b
486	32.0a	24.0a	100b	100b

Analysis of variance ($n = 50$) with arcsine transformation. Values given are transformed means. Means in a column followed by the same letter are not significantly different ($P > 0.05$; Duncan's [1955] multiple range test).

The results from the adult irradiation were difficult to interpret because control mortality was erratic (Table 4). One week after treatment, all treated adults were dead, and control mortality was <42%. Progeny were not detected in the rearing jars containing irradiated adults, but untreated adults produced large numbers of adult progeny.

Brower et al. (1973) noted that the corn sap beetle was relatively radiosensitive when compared with other species of stored product beetles. My study suggests that the DFB is also radiosensitive, with relatively rapid mortality of adult beetles occurring after irradiation with 338 Gy. Lethal doses given by Papadopoulou (1964) for DFB larvae and adults were much higher (1.0–1.50 kGy) because only a few days were allowed for complete mortality to occur. If delayed mortality is acceptable for control, a dose of 300 Gy should be effective. Doses between 300 and 400 Gy have been suggested to control the Indianmeal moth, *Plodia interpunctella* (Hübner), in dried fruit (Brower & Tilton 1970, Johnson & Vail 1987). As a group, beetles are more radiosensitive than lepidopterous species (Tilton & Burditt 1983). Consequently, in the development of radiation as a disinfestation treatment for dried fruit, any dose that proves efficacious for the Indianmeal moth should also control the DFB.

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